Phenotypic Changes That TCR Vγ3$^+$ Fetal Thymocytes Undergo During Their Maturation into Dendritic Epidermal T Cells

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Murine Thy-1$^+$, TCR Vγ3/Vδ1$^+$ dendritic epidermal T cells (DETC) express CD2 antigens, but differ from most other T-cell subsets in their absence of CD4, CD5, and CD8 antigens. To determine whether negativity for those antigens is an intrinsic feature of a given T-cell population or whether such triple-negative T cells go through a maturational stage during which they express these antigens, we determined the phenotype of TCR Vγ3$^+$ fetal thymocytes, which are the precursor cells of DETC. We found that TCR Vγ3$^+$ fetal thymocytes at day 17 of gestation are CD2$^+$, CD5$^+$, mostly CD8$^+$, and partly CD4$^+$. The expression of CD5 is highest on early TCR Vγ3$^+$ thymocytes; these cells express intermediate levels of CD5 when they leave the thymus and lose CD5 expression until or shortly after arrival in the epidermis. A similar loss of CD5 expression by TCR Vγ3$^+$ cells was observed *in vitro* under various culture conditions. To determine whether expression of CD5 is important for the maturation of DETC, we searched for these cells in the epidermis of CD5-deficient mice. There was no alteration in the number of Thy-1$^+$/TCR Vγ3$^+$ dendritic cells in the epidermis of CD5$^{-/-}$ mice. Even though the latter finding speaks against a pivotal role of CD5 during the maturation of DETC, the described cell system may serve as a useful tool in further experiments aimed to clarify the function of the CD5 glycoprotein as well as the mechanism(s) regulating its expression. *Key words: CD5/CD2/TCR γ/δ. J Invest Dermatol 105:54S–57S, 1995*

CHARACTERIZATION AND ONTOGENY OF DENDRITIC EPIDERMAL T CELLS (DETC)

The murine epidermis contains two populations of bone-marrow–derived dendritic cells, major histocompatibility complex (MHC) class II$^+$ Langerhans cells and Thy-1$^+$ dendritic epidermal T cells (DETC or Thy-1$^+$ DEC). The presence of dendritic Thy-1$^+$ cells in the epidermis was first reported in 1983. Besides Thy-1, these cells express CD45 and asialo-GM1 antigens, but they are negative for CD5, CD4, CD8, and MHC class II antigens [1,2]. Because of this phenotype, it was not clear whether these cells are T cells or natural killer cells. The discovery of a TCR γ/δ on their surface 4 years later finally established their T-cell lineage [3,4]. However, they are very unusual T cells. T cells in peripheral organs such as lymph nodes and spleen express a wide variety of different TCR (mostly TCR α/β, with a few cells expressing TCR γ/δ, both with a variety of different α and β or γ and δ chains), thereby recognizing a wide range of different antigens. DETC, in contrast, almost uniformly express TCR Vγ3/Vδ1 with identical (canonical) sequences [5,6]. The epidermis is the only tissue in adult mice that harbors TCR Vγ3 cells [5]. Their lack in the thymus of adult mice and the observation that adult bone-marrow–chimeric animals do not generate DETC [7] suggested that they have maturational requirements different from those of other peripheral T cells. A very important step in understanding the ontogeny of DETC was the finding that the thymus from fetuses at 14–18 d of gestation contains cells expressing exactly the same TCR Vγ3/Vδ1 as DETC, which suggests that these thymocytes may be DETC precursor cells [5]. Indeed, transplantation of fetal thymuses or injection of fetal thymic suspensions into congenic adult mice resulted in the appearance of donor-type DETC in the recipients' epidermis. In contrast, transplantation of thymuses from newborn mice, or the injection of thymocytes from adult mice or of fetal thymocytes depleted of TCR Vγ3$^+$ cells, did not lead to the generation of DETC [8,9]. These data clearly showed that TCR Vγ3$^+$ fetal thymocytes are the actual DETC precursor cells. Further research indicated that only stem cells from the fetal liver have the capacity to mature into TCR Vγ3$^+$ cells and that the fetal thymic milieu is crucial for the development of these cells [10,11]. The maturation of T cells involves a whole cascade of different steps, as has been shown for TCR α/β cells (reviewed in [12]). It has been known since the late 1980s that most, if not all, thymocytes acquire CD5 and CD2 antigens early in development and go through a phase of CD4 and CD8 coexpression before they mature into CD4 and CD8 single-positive T cells [12–14]. In contrast, the developmental stages that TCR γ/δ cells undergo (especially in the fetal period) were not known, and became a subject of research only recently [15,16]. Given that DETC are CD5$^-$, CD4$^-$, and CD8$^-$, we studied the expression of these molecules on their fetal thymic precursor cells. In addition, because it was not known whether negativity for CD5 is a stable trait of a given cell, or if CD5$^-$ T cells (DETC, a CD5$^-$ subpopulation of intraepithelial lymphocytes [17] and a few CD5$^-$ human peripheral blood T cells [18]) go through a stage of CD5 expression

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Abbreviation: PE-SA, phycoerythrin-streptavidin.

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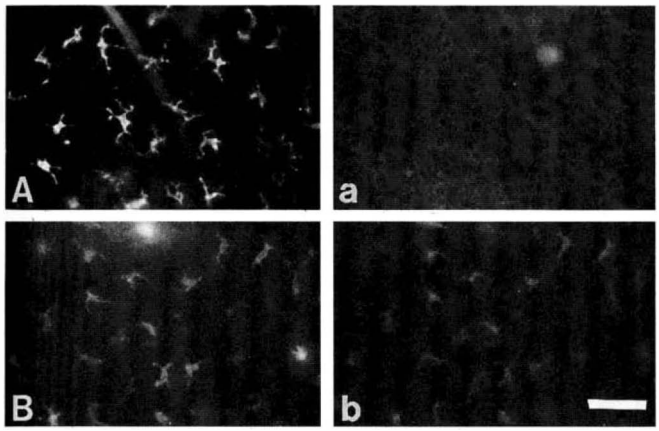

Figure 1. Immunofluorescence double labeling of epidermal ear sheets prepared from adult B6PL mice by the NH_4SCN separation technique. After acetone fixation, the sheets were incubated with biotinylated anti-CD2 or anti-CD5 MoAb, followed by Texas Red–streptavidin and FITC-labeled anti-TCR Vγ3 MoAb. The TCR $V\gamma3^+$ DETC (*A,B*) are $CD2^+$ (*b*) but consistently $CD5^-$ (*a*). *Bar*, 50 μm.

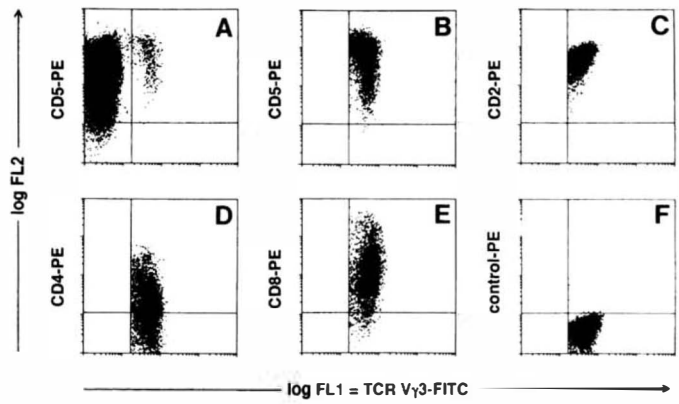

Figure 2. Flow cytometric analysis of TCR $V\gamma3^+$ fetal thymocytes at day 17 of gestation. B6PL fetal thymocytes were incubated with biotinylated anti-CD5 (*A,B*), anti-CD2 (*C*), anti-CD4 (*D*), anti-CD8 (*E*), or control (*F*) MoAb, followed by PE-SA and FITC-labeled anti-TCR Vγ3 MoAb. A total of 10,000 viable cells were recorded (*A*). Electronic gates were then set to acquire at least 5000 TCR $V\gamma3^+$ viable cells (*B–F*). All TCR $V\gamma3^+$ cells expressed CD5 (*A,B*) and CD2 (*C*) antigens, approximately half expressed CD4 antigens (*D*), and 90% expressed CD8 antigens (*E*).

during their development, we studied the regulation of CD5 cell-surface expression.

PHENOTYPIC DIFFERENCES BETWEEN TCR $V\gamma3^+$ FETAL THYMOCYTES AND DETC

To compare the phenotype of DETC with that of their intrathymic precursor cells, we first reevaluated the phenotype of DETC. Immunofluorescence analysis of resident DETC in epidermal sheet preparations as well as flow cytometric analysis of freshly prepared DETC-enriched epidermal single-cell suspensions did not reveal any detectable expression of CD5, CD4, or CD8 antigens on TCR $V\gamma3^+$ cells (**Fig 1** and data not shown). In contrast, we found that resident **(Fig 1)** as well as freshly isolated DETC [19] expressed CD2, a glycoprotein expressed on all T cells and thymocytes studied to date [14]. Three ligands for CD2 have been identified: CD58 (LFA3), CD59, and CD48 [20–22]. It remains to be seen whether the interaction between CD2 and its ligands expressed on Langerhans cells and keratinocytes [23,24], in addition to homotypic E-cadherin interactions (E-cadherins are expressed on Langerhans cells, keratinocytes [25], and DETC; M. Udey, personal communication), helps to secure the intraepithelial position of DETC.

Next we investigated the expression of the above antigens on the intrathymic DETC precursor cells at day 17 of gestation. TCR $V\gamma3^+$ fetal thymocytes showed uniform expression of CD2 antigens **(Fig 2*C*)** and also reacted with anti-CD5 monoclonal antibody (MoAb) **(Fig 2*A,B*)**. Furthermore, most of these thymocytes were $CD8^+$ **(Fig 2*E*)**, and approximately 50% of them expressed low levels of CD4 antigens **(Fig 2*D*)**. In summary, both our data and the work by Leclercq *et al* [15] show that both TCR $V\gamma3^+$ fetal thymocytes and DETC express CD2 antigens, but that these cells differ with respect to CD5, CD4, CD8, heat-stable antigen, and MEL-14 (L-selectin) expression. All of the latter antigens are present in the thymus but absent on DETC.

INTENSITY OF CD5 EXPRESSION DURING MATURATION OF TCR $V\gamma3^+$ CELLS

The transition from $CD5^+$/TCR $V\gamma3^+$ fetal thymocytes to $CD5^-$/TCR $V\gamma3^+$ DETC was subsequently studied in more detail. Intravenous injection of fetal thymocyte suspensions containing TCR $V\gamma3^+/CD5^+$ (but not TCR $V\gamma3^+/CD5^-$) fetal thymocytes into athymic nude mice resulted in the appearance of numerous clusters of donor-type $CD5^-$/TCR $V\gamma3^+$ DETC in the recipients' epidermis. We observed that even the earliest donor-derived cells, which could be visualized in the epidermis 2 weeks after intravenous cell transfer, were $CD5^-$; this indicates that the immediate DETC precursor is TCR $V\gamma3^+/CD5^-$. These cells could either be TCR $V\gamma3^+/CD5^-$ during their entire development (unlikely in view of the results presented in **Fig 2**) or could have lost CD5 expression intrathymically. Because one might postulate that TCR $V\gamma3^+$ thymocytes down-regulate CD5 expression at the end of gestation, we studied their anti-CD5 reactivity from 15 d of gestation until 2 d postpartum. **Figure 3** shows that CD5 expression was highest on TCR $V\gamma3^+$ cells at 15 d of gestation. Although at day 16 and thereafter, a fraction of TCR $V\gamma3^+$ cells had a reduced CD5 cell-surface density **(Fig 3)**, appreciable numbers of $CD5^-$ cells were never detected [19]. This suggests that TCR $V\gamma3^+$ cells leave the thymus at a stage of intermediate CD5 expression density. Analysis of CD5 expression on TCR $V\gamma3^+$ cells in fetal blood, dermis, and epidermis (day 17 of gestation) showed that the level of CD5 expression further decreased from the thymus to the epidermis **(Table I)**. CD5 ultimately became undetectable on epidermal TCR $V\gamma3^+$ cells at day 19 of gestation. These data, in conjunction with other findings—that release of DETC from their epidermal symbionts does not lead to CD5 expression [26] and that culture of freshly isolated DETC in phorbol myristate acetate (PMA) and ionomycin (PMA up-regulates CD5 expression on T

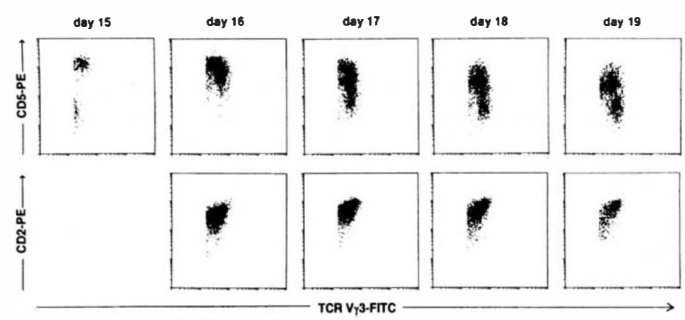

Figure 3. Flow cytometric analysis of CD5 and CD2 expression on TCR $V\gamma3^+$ thymocytes obtained at 15–19 d of gestation. Fetal thymocyte suspensions were labeled with biotinylated anti-CD5 or anti-CD2 MoAb/PE-SA and FITC-labeled anti-TCR Vγ3 MoAb, and only FITC-labeled cells were acquired. CD5 expression was down-regulated on TCR $V\gamma3^+$ cells (*lane 1*), whereas CD2 expression stayed constant (*lane 2*).

Table I. Intensity of CD5 Expression on TCR Vγ3$^+$ Cells in Various Tissues

Age of the Animal	Tissue	MFI CD5[a]	MFI Control
Day 17 fetal	Thymus	15	1.6
Day 17 fetal	Blood	11	1.8
Day 17 fetal	Dermis	6.4	1.8
Day 17 fetal	Epidermis	4.9	2.1
Day 18 fetal	Epidermis	3.9	2
Day 19 fetal	Epidermis	2	2
8–12 Weeks	Epidermis	3.3	3

[a] Single-cell suspensions were prepared from the indicated organs and reacted with biotinylated anti-CD5 or control MoAb followed by fluorescein isothiocyanate (FITC)-conjugated anti-TCR Vγ3 MoAb and phycoerythrin-streptavidin (PE-SA). CD5 (or control) fluorescence intensity was measured with a flow cytometer on gated TCR Vγ3$^+$ cells using logarithmic amplification. Data are given as the mean fluorescence intensity (MFI) of the respective staining.

cells [27]) also does not lead to CD5 expression (data not shown)—indicate that the absence of CD5 molecules on DETC is not due to suppressive signals provided by other epidermal cells.

CD5 EXPRESSION ON TCR Vγ3$^+$ THYMOCYTES *IN VITRO*

To understand the mechanism(s) regulating CD5 cell-surface density on DETC precursor cells, we studied CD5 expression on thymocytes isolated from fetuses at day 17 of gestation and cultured under different conditions. Independent of whether these cells were maintained in plain medium, in medium supplemented with concanavalin A plus 10 U IL-2, or in medium supplemented with 100 U IL-2 (a condition leading to the preferential proliferation of TCR Vγ3$^+$ cells [28]), or were studied in intact thymic lobe explants, a complete or at least substantial loss of CD5 antigens on TCR Vγ3$^+$ cells always occurred within 6–8 d [19]. Even the addition of PMA and ionomycin to the cultures could not prevent the down-regulation of CD5 on these special thymocytes **(Table II)**. This was surprising because PMA enhances CD5 expression on lymph node T cells [27] as well as on TCR-α/β–expressing fetal thymocytes **(Table II)**. Because we were unable to up-regulate CD5 expression on TCR Vγ3$^+$ fetal thymocytes and because sorted TCR γ/δ fetal thymocytes lost CD5 expression (ruling out down-regulatory signals delivered from other thymic cell populations), we conclude that TCR Vγ3$^+$ thymocytes may have an intrinsic program to down-regulate CD5 and finally to lose expression of this antigen completely.

MATURATION OF DETC IN CD5-DEFICIENT MICE

The patterns of expression of CD5, CD8, and CD4 molecules within the thymus led to the speculation that these molecules play a role in the maturation and/or selection of these cells. We tested this hypothesis with respect to the CD5 molecule by studying the

Table II. Intensity of CD5 Expression After *In Vitro* Culture of Fetal Thymocytes in Medium Supplemented With PMA and Ionomycin

Cell Population	Day 0	Day 3	Day 7
TCR Vγ3$^+$ FT[a]	382 ± 116	295 ± 123	72 ± 15
TCR α/β$^+$ FT[a]	226 ± 25	1065 ± 486	577 ± 225
TCR α/β$^+$ LN[b]	965 ± 262	3462 ± 327	3029 ± 764

[a] Single-cell suspensions of fetal thymocytes (FT) obtained at day 17 of gestation were cultured for the indicated period in normal culture medium supplemented with 10 ng/ml PMA and 500 ng/ml ionomycin. Cells were then labeled with biotinylated anti-CD5/PE-SA and FITC-labeled anti-TCR Vγ3 or anti-pan TCR α/β MoAb. Samples were analyzed on a flow cytometer, and the mean fluorescence intensity of the anti-CD5 staining was recorded on gated FITC-positive cells. Mean values ± SD of three independent experiments are shown.

[b] Single-cell suspensions were prepared from lymph nodes (LN) obtained from 6–8-week B6PL mice. Cells were treated, stained, and analyzed as described in *a*.

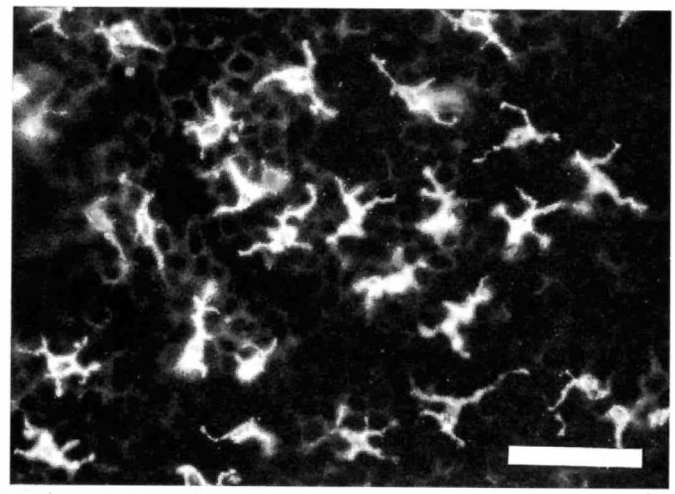

Figure 4. Immunofluorescence detection of TCR Vγ3$^+$ cells in the epidermis of a CD5-deficient C57BL/6 mouse. Epidermal sheets were incubated with biotinylated anti-TCR Vγ3 MoAb, followed by Texas Red-streptavidin. Note that there is no difference in the shape or the density of the DETC in the epidermis of the CD5-deficient mouse compared with normal C57BL/6 (not shown) or B6PL mice **(Fig 1)**. *Bar*, 50 μm.

DETC population in CD5-deficient mice [29]. Tarakhovsky *et al* [29] used the approach of homologous recombination to disrupt the CD5 gene in a way that prevents the appearance of the CD5 antigen on the cell surface. Mutant mice developed normally, and the numbers and phenotypes of T and B cells in the bone marrow, spleen, lymph nodes, and peritoneum of these mice matched those of normal control mice [29]. We prepared epidermal sheets of these mice and studied their reactivity with anti-Thy-1 and anti-TCR Vγ3 MoAb. As can be seen in **Fig 4**, there was no alteration in the appearance and density of TCR Vγ3$^+$ cells in the CD5-deficient mice compared with normal mice (see **Fig 1**), and all Thy-1$^+$ cells coexpressed TCR Vγ3. The possibility that DETC in CD5-deficient mice have a functional defect cannot be excluded, but appears quite unlikely in view of studies by Tarakhovsky *et al* [29] showing no alterations in the immune function of their mice. Although these data argue against an important role for CD5 in the maturation of DETC, other T cells, and B cells, it is conceivable that in the setting of knockout animals, other molecules may have substituted for the function of the CD5 molecules, and alterations may become visible only after the inactivation of a combination of cell-surface markers.

Similar to the situation in CD5-deficient mice, no alterations in the appearance of DETC or other TCR γ/δ cells have been observed in mice in which the interactions of CD4 or CD8 antigens with MHC class II or class I were interrupted as a result of inactivation of the latter genes [30,31]. This is not surprising because the intriguing TCR homogeneity of these cells is generated by an intrinsic program of the recombinatory machinery rather than from selection [32,33].

CONCLUDING REMARKS

Our study of TCR Vγ3$^+$ fetal thymocytes as well as the studies from Leclercq *et al* [15] and Tatsumi *et al* [16] show that TCR γ/δ fetal thymocytes undergo several phenotypic changes during their maturation in the thymus. Some of these changes are similar to the changes of TCR α/β thymocytes, e.g., the early expression of heat-stable antigen and loss of this molecule on mature cells [15,16]. With respect to CD4 and CD8 molecules, there are slight differences between TCR α/β and TCR γ/δ thymocytes. Although the latter possess some anti-CD4 and anti-CD8 reactivity [34,35], they apparently do not go through a stage of high CD4/CD8 double expression. Although this stage is important for the positive and negative selection of TCR α/β cells, selection of TCR γ/δ

cells (if it occurs at all) does not require this stage. However, the regulation of CD5 on TCR $V\gamma3^+$ fetal thymocytes contrasts sharply with that of TCR α/β cells. Whereas the latter population enhances the level of CD5 cell-surface density during maturation [19], TCR $V\gamma3^+$ thymocytes start with high CD5 levels and then lose this antigen. The functional consequence(s) of these changes is unclear, especially because CD5-deficient mice generate apparently normal peripheral and epidermal T cells. We therefore assume that intrathymic expression of this glycoprotein on DETC precursor cells is not important for the maturation of these cells, but rather propose that the absence of this molecule on DETC is important for their function. CD5 is associated with the TCR complex and can deliver stimulatory signals synergizing with TCR activation [36–39]. In this context, it should be noted that the $CD5^-$ DETC as well as the TCR γ/δ, $CD5^-$, $CD2^-$ subpopulation of intraepithelial lymphocytes exhibit a reduced proliferation in response to mitogens in the absence of exogenously added IL-2 [17,40,41]. One could therefore speculate that the absence of CD5 on these cells increases the threshold level of antigen to which the cells respond, ensuring that these cells become activated only when the epidermis is damaged [42] or the bacterial load of the gut is enhanced [43], and not by low levels of antigen conceivably present in "normal" skin and gut. The cellular system described in this article and DETC transfected with CD5 may serve as useful tools for experiments designed to test the proposed hypothesis and unravel the role of CD5.

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REFERENCES

1. Tschachler E, Schuler G, Hutterer J, Leibl H, Wolff K, Stingl G: Expression of Thy-1 antigen by murine epidermal cells. *J Invest Dermatol* 81:282–285, 1983
2. Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW: Thy-1 antigen-bearing dendritic cells populate murine epidermis. *J Invest Dermatol* 81:286–288, 1983
3. Stingl G, Koning F, Yamada H, Yokoyama WM, Tschachler E, Bluestone JA, Steiner G, Samelson LE, Lew AM, Coligan JE: Thy-1^+ dendritic epidermal cells express T3 antigen and the T-cell receptor γ chain. *Proc Natl Acad Sci USA* 84:4586–4590, 1987
4. Steiner G, Koning F, Elbe A, Tschachler E, Yokoyama WM, Shevach EM, Stingl G, Coligan JE: Characterization of T cell receptors on resident murine dendritic epidermal T cells. *Eur J Immunol* 18:1323–1328, 1988
5. Havran WL, Allison JP: Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature* 335:443–445, 1988
6. Asarnow DM, Goodman T, LeFrancois L, Allison JP: Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells. *Nature* 341:60–62, 1989
7. Honjo M, Elbe A, Steiner G, Assmann I, Wolff K, Stingl G: Thymus-independent generation of Thy-1^+ epidermal cells from a pool of Thy-1^- bone marrow precursors. *J Invest Dermatol* 95:562–567, 1990
8. Havran WL, Allison JP: Origin of Thy-1^+ dendritic epidermal cells of adult mice from fetal thymic precursors. *Nature* 344:68–70, 1990
9. Payer E, Elbe A, Stingl G: Circulating $CD3^+$/T cell receptor $V\gamma3^+$ fetal murine thymocytes home to the skin and give rise to proliferating dendritic epidermal T cells. *J Immunol* 146:2536–2543, 1991
10. Ikuta K, Kina T, MacNeil I, Uchida N, Peault B, Chien YH, Weissman IL: A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. *Cell* 62:863–874, 1990
11. Allison JP, Havran WL: The immunobiology of T cells with invariant $\gamma\delta$ antigen receptors. *Annu Rev Immunol* 9:679–705, 1991
12. Shortman K: Cellular aspects of early T-cell development. *Curr Opin Immunol* 4:140–146, 1992
13. Mathieson BJ, Sharrow SO, Rosenberg Y, Hammerling U: Lyt 1^+23^- cells appear in the thymus before Lyt 123^+ cells. *Nature* 289:179–181, 1981
14. Duplay P, Lancki D, Allison JP: Distribution of CD2 expression by murine T cells. *J Immunol* 142:2998–3005, 1989
15. Leclercq G, Plum J, Nandi D, De Smedt M, Allison JP: Intrathymic differentiation of $V\gamma3$ T cells. *J Exp Med* 178:309–315, 1993.
16. Tatsumi Y, Pena JC, Matis L, Deluca D, Bluestone JA: Development of T cell receptor-$\gamma\delta$ cells. *J Immunol* 151:3030–3041, 1993
17. LeFrancois L: Phenotypic complexity of intraepithelial lymphocytes of the small intestine. *J Immunol* 147:1746–1751, 1991
18. Srour EF, Leemhuis T, Jenski L, Redmond R, Fillak D, Jansen J: Characterization of normal human $CD3^+$ $CD5^-$ and γ/δ T cell receptor positive T lymphocytes. *Clin Exp Immunol* 80:114–121, 1990
19. Payer E, Kutil R, Stingl G: $CD5^-$ dendritic epidermal T cells are derived from $CD5^+$ precursor cells. *Eur J Immunol* 24:1317–1322, 1994
20. Springer TA, Dustin ML, Kishimoto TK, Marlin SD: The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. *Annu Rev Immunol* 5:223–252, 1987
21. Deckert M, Kubar J, Zoccola D, Bernard-Pomier G, Angelisova P, Horejsi V, Bernard A: CD59 molecule: a second ligand for CD2 in T cell adhesion. *Eur J Immunol* 22:2943–2947, 1992
22. Kato K, Koyanagi M, Okada H, Takanashi T, Wong YW, Williams AF, Okumura K, Yagita H: CD48 is a counter-receptor for mouse CD2 and is involved in T cell activation. *J Exp Med* 176:1241–1249, 1992
23. De Panfilis G, Manara GC, Ferrari C, Torresani C: Adhesion molecules on the plasma membrane of epidermal cells. III. Keratinocytes and Langerhans cells constitutively express the lymphocyte function-associated antigen 3. *J Invest Dermatol* 96:512–517, 1991
24. Sayama K, Shiraishi S, Shirakata Y, Kobayashi Y, Okada N, Okada H, Miki K: Characterization of homologous restriction factor (HRF20) in human skin and leukocytes. *Clin Exp Immunol* 82:355–358, 1990
25. Tang A, Amagai M, Granger LG, Stanley JR, Udey MC: Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. *Nature* 361:82–85, 1993
26. Elbe A, Kilgus O, Strohal R, Payer E, Schreiber S, Stingl G: Fetal skin: a site of dendritic epidermal T cell development. *J Immunol* 149:1694–1701, 1992
27. Vandenberghe P, Ceuppens JL: Immobilized anti-CD5 together with prolonged activation of protein kinase C induce interleukin 2-dependent T cell growth: evidence for signal transduction through CD5. *Eur J Immunol* 21:251–259, 1991
28. Leclercq G, De Smedt M, Tison B, Plum J: Preferential proliferation of T cell receptor $V\gamma3$-positive cells in IL-2-stimulated fetal thymocytes. *J Immunol* 145:3992–3997, 1990
29. Tarakhovsky A, Mueller W, Rajewsky K: Lymphocyte populations and immune responses in CD5-deficient mice. *Eur J Immunol* 24:1678–1684, 1994
30. Bigby M, Markowitz JS, Bleicher PA, Grusby MJ, Simha S, Siebrecht M, Wagner M, Nagler-Aderson C, Glimcher LH: Most $\gamma\delta$ T cells develop normally in the absence of MHC class II molecules. *J Immunol* 151:4465–4475, 1993
31. Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R: $\beta2$-microglobulin deficient mice lack $CD4^-8^+$ cytolytic T cells. *Nature* 344:742–746, 1990
32. Itohara S, Mombaerts P, Lafaille J, Iacomini J, Nelson A, Clarke AR, Hooper ML, Farr A, Tonegawa S: T cell receptor δ gene mutant mice: independent generation of $\alpha\beta$ T cells and programmed rearrangements of $\gamma\delta$ TCR genes. *Cell* 72:337–348, 1993
33. Asarnow DM, Cado D, Raulet DH: Selection is not required to produce invariant T-cell receptor γ-gene junctional sequences. *Nature* 362:158–160, 1993
34. Fisher AG, Ceredig R: $\gamma\delta$ T cells expressing CD8 or $CD4^{low}$ appear early in murine foetal thymus development. *Int Immunol* 3:1323–1328, 1991
35. Itohara S, Nakanishi N, Kanagawa O, Kubo R, Tonegawa S: Monoclonal antibodies specific to native murine T-cell receptor $\gamma\delta$: analysis of $\gamma\delta$ T cells during thymic ontogeny and in peripheral lymphoid organs. *Proc Natl Acad Sci USA* 86:5094–5098, 1989
36. Beyers AD, Spruyt LL, Williams AF: Molecular associations between the T-lymphocyte antigen receptor complex and the surface antigens CD2, CD4, or CD8 and CD5. *Proc Natl Acad Sci USA* 89:2945–2949, 1992
37. Osman N, Ley SC, Crumpton MJ: Evidence for an association between the T cell receptor/CD3 antigen complex and the CD5 antigen in human T lymphocytes. *Eur J Immunol* 22:2995–3000, 1992
38. Ledbetter JA, June CH, Grosmaire LS, Rabinovitch PS: Crosslinking of surface antigens causes mobilization of intracellular ionized calcium in T lymphocytes. *Proc Natl Acad Sci USA* 84:1384–1388, 1987
39. Ceuppens JL, Baroja ML: Monoclonal antibodies to the CD5 antigen can provide the necessary second signal for activation of isolated resting T cells by solid-phase-bound OKT3. *J Immunol* 137:1816–1821, 1986
40. Tschachler E, Steiner G, Yamada H, Elbe A, Wolff K, Stingl G: Dendritic epidermal T cells: activation requirements and phenotypic characterization of proliferating cells. *J Invest Dermatol* 92:763–768, 1989
41. Van Houten N, Mixter PF, Wolfe J, Budd RC: CD2 expression on murine intestinal intraepithelial lymphocytes is bimodal and defines proliferative capacity. *Int Immunol* 5:665–672, 1993
42. Yamamoto S, Russ F, Teixeira HC, Conradt P, Kaufmann SHE: *Listeria monocytogenes*-induced gamma interferon secretion by intestinal intraepithelial γ/δ T lymphocytes. *Infect Immun* 61:2154–2161, 1993
43. Havran WL, Chien YH, Allison JP: Recognition of self antigens by skin-derived T cells with invariant $\gamma\delta$ antigen receptors. *Science* 252:1430–1432, 1991